AD_____

Award Number: DAMD17-02-1-0263

TITLE: Interstitial Metabolic Monitoring During Hemorrhagic Shock

PRINCIPAL INVESTIGATOR: Motilal B. Pamnani, M.B.B.S., Ph.D.

CONTRACTING ORGANIZATION: Henry M. Jackson Foundation for the Advancement of Military Medicine
Rockville, Maryland 20852-1428

REPORT DATE: April 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2004	Annual (15 Mar 2003 - 14 Mar 2004)

4. TITLE AND SUBTITLE
Interstitial Metabolic Monitoring During Hemorrhagic Shock

5. FUNDING NUMBERS
DAMD17-02-1-0263

6. AUTHOR(S)
Motilal B. Pamnani, M.B.B.S., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Henry M. Jackson Foundation for the Advancement of Military Medicine
Rockville, Maryland 20852-1428

E-Mail: mpamnani@usuhs.mil

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Decompensation in hemorrhagic shock is the critical stage after which resuscitative efforts may prove futile. We hypothesize that decompensation results from potassium-mediated vasodilation and/or loss of cardiac contractility, and thus a method of measuring interstitial potassium should be a crucial part of future metabolic monitoring efforts. Anesthetized rats underwent controlled hemorrhage to a constant mean arterial pressure of 40 mmHg. Microdialysis probes were implanted in skeletal muscle, vein, and liver for continuous assessment of potassium, glucose, lactate, pyruvate, and glycerol concentrations. Arterial blood samples were drawn at 30-minute intervals. Animals were sacrificed in early (pre-decompensatory) and late (decompensatory) hemorrhagic shock and tissues analyzed for ex vivo Na^+,K^+-ATPase activity. Potassium concentrations in muscle interstitium were significantly higher in hemorrhaged animals than controls (2.34 times baseline vs. 1.24, $p < 0.05$), this difference was not reflected in blood values. Na^+,K^+-ATPase activity in late hemorrhagic shock was increased vs. controls ($p< 0.05$) in kidney, skeletal muscle, cardiac muscle, diaphragm, and red blood cells. ATPase activity was also increased in early hemorrhagic shock in skeletal and cardiac muscle. These data may provide clues into new ways to monitor and treat victims of hemorrhagic shock on the battlefield.

14. SUBJECT TERMS
Shock, hemorrhage, decompensation, potassium, electrolytes, interstitium, microdialysis

15. NUMBER OF PAGES
19

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

Decompensation in hemorrhagic shock is the critical stage after which resuscitative efforts may prove futile. We hypothesize that decompensation results from potassium-mediated vasodilation and/or loss of cardiac contractility, and thus a method of measuring interstitial potassium should be a crucial part of future metabolic monitoring efforts. Anesthetized rats underwent controlled hemorrhage to a constant mean arterial pressure of 40 mmHg. Microdialysis probes were implanted in skeletal muscle, vein, and liver for continuous assessment of potassium, glucose, lactate, pyruvate, and glycerol concentrations. Arterial blood samples were drawn at 30-minute intervals. Animals were sacrificed in early (pre-decompensatory) and late (decompensatory) hemorrhagic shock and tissues analyzed for ex vivo Na^+,K^+-ATPase activity. Potassium concentrations in muscle interstitium were significantly higher in hemorrhaged animals than controls (2.34 times baseline vs. 1.24, $p < 0.05$), this difference was not reflected in blood values. Na^+,K^+-ATPase activity in late hemorrhagic shock was increased vs. controls ($p< 0.05$) in kidney, skeletal muscle, cardiac muscle, diaphragm, and red blood cells. ATPase activity was also increased in early hemorrhagic shock in skeletal and cardiac muscle. These data may provide clues into new ways to monitor and treat victims of hemorrhagic shock on the battlefield

BODY

Details on the background and experimental method may be found in the research proposal (MRMC Log No. 01155005). The following tasks composed the Statement of Work for Year Two:

1. Refine experimental protocols as necessary.

Cardiac contractility

Cardiac contractility experiments were originally proposed to commence in March of 2003. Over the past year, we have performed pilot experiments of cardiac contractility measurement in rats and found the technique and equipment to be satisfactory. However, these measurements require significant modifications from our present protocol: In order to transduce the left ventricle, a carotid artery must be catheterized. At present we bleed the animal through a catheter in the left carotid artery. To avoid catheterizing both carotids, which will result in cerebral ischemia, we need to move the bleed catheter to the right femoral artery. The left femoral artery is currently used for transducing blood pressure. Since both legs will then have their blood flow interrupted, the now-ischemic femoral veins and hind leg muscles will no longer be suitable representative tissues for microdialysis probe placement. As such we propose to move the microdialysis probes to the pectoralis muscle and vein. Given the degree of modification required, we felt it best to delay the contractility studies until the resuscitation experiments are complete, in order that those experiments will be comparable to the non-resuscitated groups.

New HSDAQ Program

A new version of the WRAIR Hemorrhage Shock Data Acquisition (HSDAQ) Program has been developed which allows for more sophisticated blood pressure control and automated incorporation of resuscitation protocols. We have purchased a new computer and data acquisition boards and implemented the new version of the program into our protocol.

ICP-MS measurements of Ca^{2+} and Mg^{2+}

Inductively-coupled plasma mass spectrometry (ICP-MS) is the method used to analyze electrolytes concentrations from the microdialysis samples. As described in the research proposal, we infuse a perfusate solution of Rb, ^{44}Ca, and ^{26}Mg and measure their relative loss compared to uptake of K, ^{40}Ca, and ^{24}Mg. We have successfully measured K and Rb concentrations in the range of 100-400 ppb (approx. 1000x dilution from physiologic levels). Measurement of Mg and especially Ca concentrations and isotopic ratios has been complicated because of interference from the Argon needed as a carrier gas for the samples. We have spent considerable time and resources over the past year towards reducing those interferences.

We have consulted extensively with the laboratory of Prof. William McDonough at the University of Maryland on the "cold plasma" ICP-MS technique and the use of microflow nebulizers as front-end methods for improving sensitivity. With the use of these techniques we are now able to consistently measure Mg concentrations and isotopic ratios in the desired range. Measurement of ^{40}Ca remains problematic even with these more sophisticated techniques, and may not be achievable at the desired sensitivity. We are now exploring the measurement of an alternative, less-abundant natural Ca isotope such as ^{42}Ca, ^{43}Ca, or ^{48}Ca as the surrogate for the tissue concentrations.

Our work this past year was also affected by move of the Department of Clinical Investigation laboratories at Walter Reed Army Medical Center in September 2003, which necessitated shutting down, relocating, setting up, and recalibration of the ICP-MS equipment. In total, the equipment was off-line for a period of about three months.

CMA 600 measurements

In March 2003 we purchased a CMA 600 microdialysis analyzer for measurement of glucose, lactate, pyruvate, and glycerol concentrations from microdialysis samples. Soon after its arrival analyzer started functioning erratically, resulting in several weeks of troubleshooting by CMA technicians. Eventually the motherboard became had to be replaced.

Because of our small sample sizes (~5 μl) and the relative heat of the CMA 600 sample chamber, we became concerned about the possibility of significant evaporative losses during the analysis time (usually around 2-3 hours). As such we modified our technique to include dilution of the microdialysis sample to increase the sample volume and sealing the sample tubes with a Teflon cap.

2. **Conduct experiments in 70 animals**
3. **Present analysis of correlations of interstitial concentrations to corresponding intravascular concentrations and to state of shock**

Over the past year, we have performed experiments in 56 animals, with a total number of 116 animals over two years. The reasons for the lower-than-planned number include the various technical problems mentioned previously.

Baseline data

Table 1 shows the baseline data for the animals done in the protocol under the most updated modification. There are three hemorrhage groups, one early-stage (pre-decompensatory stage) hemorrhagic shock (bled volume = 50% of predicted peak shed blood volume) and two late-stage (decompensatory stage) hemorrhagic shock (bleed volumes = 25% and 50% return of peak shed blood volume). There were corresponding time and weight-matched controls for the late shock animals.

The data subsequently plotted in Figures 1-9 does not represent all animals done to date but a subset which has been analyzed in greater detail.

Figure 1 shows average hemodynamic data (mean arterial pressure and shed blood volume) for a the late hemorrhage experiments vs. controls. The plots demonstrate how the HSDAQ program reproducibly maintains the hemorrhaged animals at the desired target pressure.

Figure 2 shows the average pH and change in base excess for the animals, providing an illustration of the severity of the shock administered by the hemorrhage

Shown in Figures 3-8 are arterial and microdialysis results for K^+ and various other metabolic parameters.. To emphasize the changes with time, most values are shown as ratios to baseline. Vertical lines represent the average time to peak shed blood volume.Potassium (Figure 3): Interstitial [K^+] was higher in muscle at peak SBV than controls (ratio = 2.33 vs. 1.24 times baseline, $p < 0.05$); this was not reflected in vein or liver. These results are consistent with previous studies (Illner and Shires, 1980; McKinley *et al.*, 1981). After peak SBV, average muscle [K^+] declines vs. time due to drop-out of animals reaching experimental end. When vein and muscle levels in hemorrhaged rats are plotted as a scattergram vs. stage of shock (% of peak SBV bled, third panel of Figure 3), the slope of the linear correlation to the muscle data is larger (1.87 vs. 1.12).

Glucose (Figure 4): Arterial glucose ratios were higher (2.68 vs. 1.06, $p < 0.05$) at peak SBV than controls, then decreased. Similar trends were seen in venous and muscle samples, but did not reach significance. In liver samples no trend versus time for either groups was appreciated.

Lactate (Figure 5): Values in all tissues were higher in hemorrhaged animals, but only reached significance in venous samples at $t = 28$ (ratio = 3.05 vs. 1.42) and 43 (6.91 vs. 1.67) minutes. In muscle similar differences were seen (ratios = 2.8 vs. 0.8 @ 28 min and 4.9 vs. 0.8 @ 43 min) but hemorrhage vs. control differences were not significant due to small $n = 4$ from analytical problems. In liver the difference at t = 28 min just fell short of statistical significance (2.74 vs. 1.32, $p = 0.068$). The smaller relative increase in muscle and liver is consistent with previous results and has been used to argue that these tissues are lactate consumers in hemorrhage (Okuda *et al.*, 1992).

Pyruvate (Figure 6): Venous samples tended to increase with time in both hemorrhage and control groups without statistically significant difference. Samples from muscle and liver in both groups showed no clear change with time.

L/P ratio (Figure 7): Trended upward with time in control liver samples and all hemorrhaged samples without significant statistical difference.

Glycerol (Figure 8): Levels in all tissues tended to increase in both hemorrhage and control animals, without significant difference between groups.

Figure 9 shows *ex vivo* tissue Na,K-ATPase results from each of the experimental groups. ATPase activity was significantly increased in late hemorrhagic shock compared to controls in all tissues with the exception of liver. The time course of this change, however, appears to be different in different tissues: In skeletal muscle and heart, the activity appears to peak in early hemorrhagic shock, whereas in kidney and diaphragm it appears to peak in the decompensatory phase. The results from early hemorrhage in red blood cells are still in-process.

The observed time variations in time of ATPase activity may be of considerable pathophysiological significance: Recently, other investigators have concluded through indirect measurements that ATPase activity increases in hemorrhagic shock, and that this increase in activity may be a mechanism of lactate production (Luchette *et al*, 1998 and 2002; McCarter *et al*, 2001). The observed shift of potassium from the intracellular to the extracellular compartment is at odds with this physiology, however. There are many potential explanations for this, however, our data suggests that one possibility may be an initial increase then a relative decrease from peak activity levels as shock progresses.

4. Transition animal experiments to include resuscitation protocols

As stated earlier, we have upgraded our experimental procedure with more advanced data acquisition technology which will allow us to automate the resuscitation step in the protocol. The resuscitation experiments will begin in the next month.

FUTURE WORK

In the next year, we will perform the studies of resuscitation in early and late hemorrhagic shock and of measurements of cardiac contractily. In addition, more experiments may be performed to fully elucidate the time course of the observed changes in Na,K-ATPase during the phases of hemorrhagic shock. Of particular interest will be correlation of contractility with cardiac Na,K-ATPase activity.

For the microdialysis studies, we will continue refinement of our analytical technique to achieve more reliable calcium measurements. An additional goal will be increasing the sensitivity of the ICP-MS technique to allow for smaller sample requirement (and correspondingly larger sample available for the CMA 600 analysis of glucose, lactate, pyruvate, and glycerol) We will also consider looking at measurement of K/Na ratios in the microdialysis fluid as a means of obviating the need for Rb as an internal standard.

None of the planned studies will require changes in the present Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS

- Interstitial hyperkalemia appears to correlate with the onset of hemodynamic decompensation in hemorrhagic shock.
- *Ex vivo* ATPase activity is increased from controls in kidney, heart, skeletal muscle, diaphragm, and red blood cells in late hemorrhagic shock (25-50% return of shed blood volume). In skeletal muscle and heart, it is also elevated in early hemorrhagic shock, suggesting the possibility that a fall from peak activity may correlate with the onset of decompensation.

REPORTABLE OUTCOMES

Published Abstracts

Oliver, III, J. D., J. L. Atkins, J. L. Schooley, E. R. Morris, L. Ma, T. B. Bentley, and M. B. Pamnani. Interstitial concentrations during hemorrhagic shock. *Shock*, **19 Suppl**:53, 2003.

Oliver III, J. D., J. L. Atkins, J. F. Schooley, L. Ma, T. B. Bentley, and M. B. Pamnani. Hemorrhagic Shock in Rats Increases Microsomal Sodium-Potassium ATPase (NKA) Activity *Ex Vivo. Shock*, **21 Suppl**:43, 2004.

Presentations

Date	Title	Meeting
15 Apr 03	Microdialysis (μD) Measurement Of Interstitial Markers of Hemorrhagic Shock	Experimental Biology San Diego, CA
09 Jun 03	Interstitial Concentrations During Hemorrhagic Shock	Shock Society Phoenix, AZ
03 Sep 03	Who's Going to Crash: Will Interstitial Potassium Tell Us In Time?	Advanced Technology Applications for Combat Casualty Care (ATACCC) St. Pete Beach, FL
06 Mar 04	Hemorrhagic Shock in Rats Increases Microsomal Sodium-Potassium ATPase (NKA) Activity *Ex Vivo*	6th World Congress on Trauma, Shock, Inflammation, and Sepsis Munich, Germany

CONCLUSIONS

[K+] in skeletal muscle during hemorrhage appears to correlate with the onset of decompensation, while intravascular [K+] does not. Changes in blood glucose also correlate with peak SBV. Muscle and liver glucose may be similarly correlated, although the magnitude of the change appears to be less. The rise in venous lactate levels from microdialysis also corresponded with peak SBV. Changes in tissue lactate had similar trends but did not reach

statistical significance due to small numbers. Interstitial measures of potassium, lactate, and/or glucose may prove to be of diagnostic and prognostic significance in hemorrhagic shock. Interstitial hyperkalemia may be a physiological mechanism for decompensation..

Hemorrhagic shock increases the *ex vivo* NKA activity in most tissues. The rise in extracellular potassium seen in hemorrhage thus does not appear to be due to a reduction in the intrinsic pump capacity. Possible explanations include the presence of an *in vivo* NKA inhibitor, an uncoupling of NKA activity from potassium transport, or through effects of other potassium pathways. The mechanism for increases in NKA activity *in vivo* may be due to stimulation from catecholamines, high extracellular potassium, or intravascular volume depletion.

Further investigation of these metabolic derangements in hemorrhagic shock may provide critical insight into ways to monitor and treat casualties on the battlefield.

REFERENCES

Illner, H and GT Shires. *Surg. Gynecol. Obstet.*, 150:17, 1980

Luchette, FA, LA Friend, *et al. J. Trauma*, 44:796, 1998.

Luchette, FA, WA Jenkins, *et al.*, *J. Trauma*, 53:807, 2002.

McCarter, FD, JH James, *et al. J. Surg. Res.*, 99:235, 2001

McKinley BA, BA Houtchens, and J. Janata. *Crit Care Med.*, 9:845, 1981.

Okuda , C, T Sawa, et al.. *Circ. Shock.*, 37:230, 1992.

Pearce, FJ and WR Drucker. *J. Trauma*, 27:1213-1220, 1987.

APPENDICES

	Early Hemorrhage	Late Hemorrhage			
	H50%pSBV	H25%SBVR	C25%SBVR	H50%SBVR	C50%SBVR
n	8	23	23	8	7
Weight (g)	437±13	397±16	398±15	362±5	367±8
MAP_0 (mmHg)	95±5	97±4	86±4	94±4	100±7
HR_0 (bpm)	342±9	379±7	367±11	365±17	405±9
pSBV (ml)	3.5±0.4*	6.1±0.4	-------	7.0±0.5	-------
t-pSBV (min)	28±4*	58±5	-------	91±7†	-------
t-bleed (min)	29±4*	95±12	-------	191±12†	-------

Table 1. Baseline hemodynamic data for early hemorrhage groups (H50%pSBV = hemorrhage to 50% of predicted peak shed blood volume) vs. late hemorrhage groups (H25%SBVR and H50%SBVR = hemorrhage to 25% and 50% return of peak shed blood volume, respectively) and corresponding time and weight-matched control groups (C25%SBVR and H50%SBVR). MAP_0 = mean arterial blood pressure at end of 20-minute control phase prior to bleed, HR_0 = heart rate at end of control phase, t-pSBV = time to peak shed blood volume from start of bleed, t-bleed = total experimental time from start of bleed. $^*p < 0.05$ for early hemorrhage vs. both late groups, $^\dagger p < 0.05$ for H50%SBVR vs. H25%SBVR.

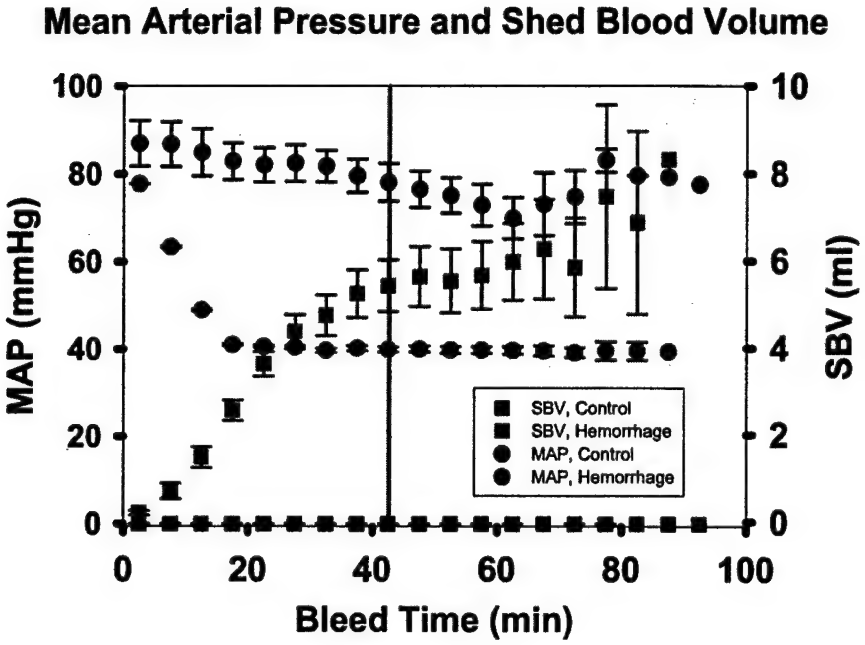

Figure 1. Average mean arterial pressure and shed blood volume for late hemorrhage (25% return of shed blood volume) animals. Vertical line represent average time to peak shed blood volume. Error bars represent ± one S.E.M.

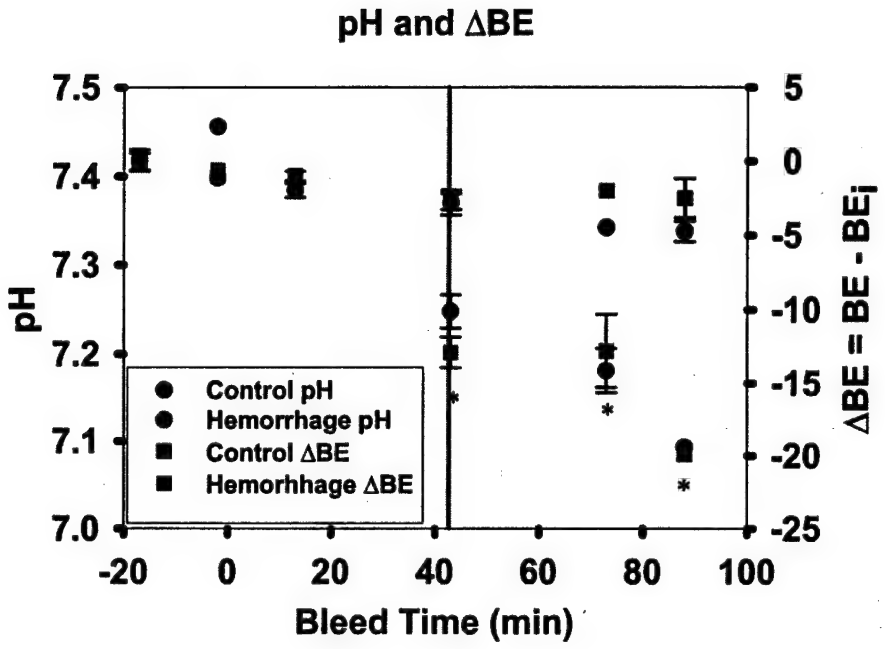

Figure 2. pH and change in base excess for hemorrhage vs. control animals. Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.

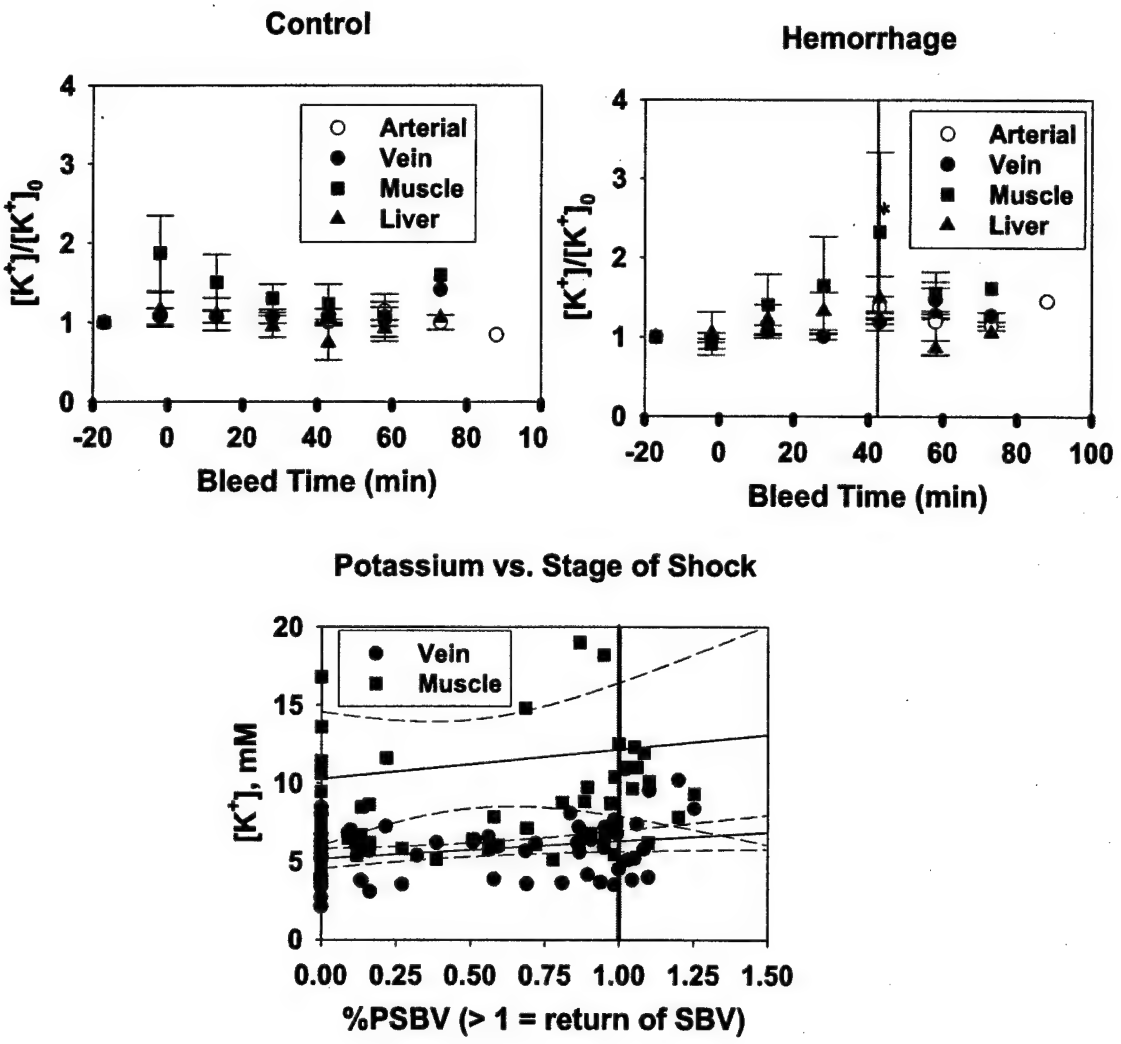

Figure 3. (Top panels) Potassium concentrations relative to baseline levels in arterial (open circles) and tissue microdialysis (closed symbols) samples (Bottom panel) Scattergram of potassium concentration vs. stage of shock (percentage bled of peak shed blood volume). Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.

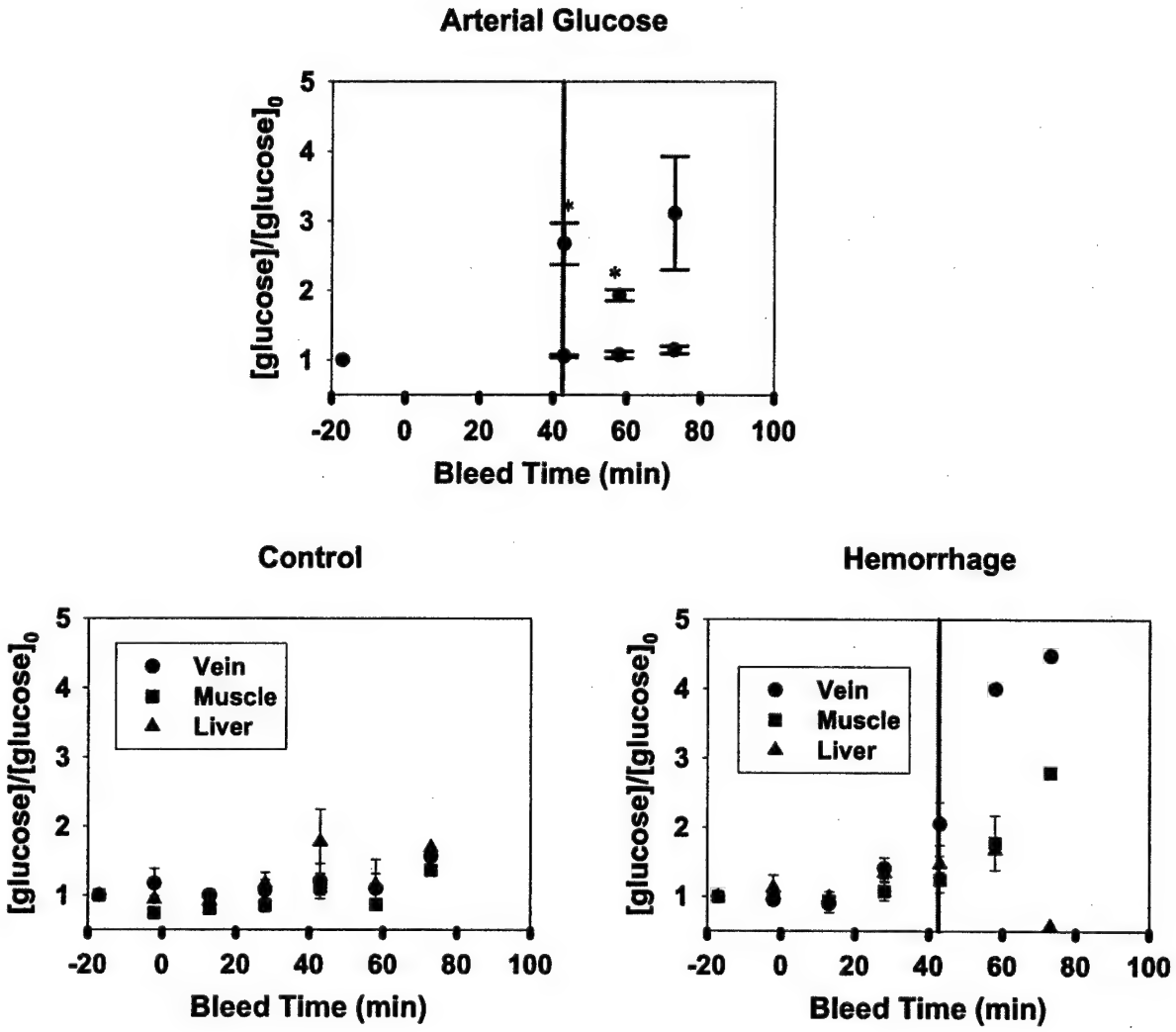

Figure 4. (Top panel) Relative arterial glucose concentrations for hemorrhage vs. controls. (Bottom panels) Relative glucose levels from microdialysis samples. Error bars represent ± one S.E.M. $*p < 0.05$ for hemorrhage versus control.

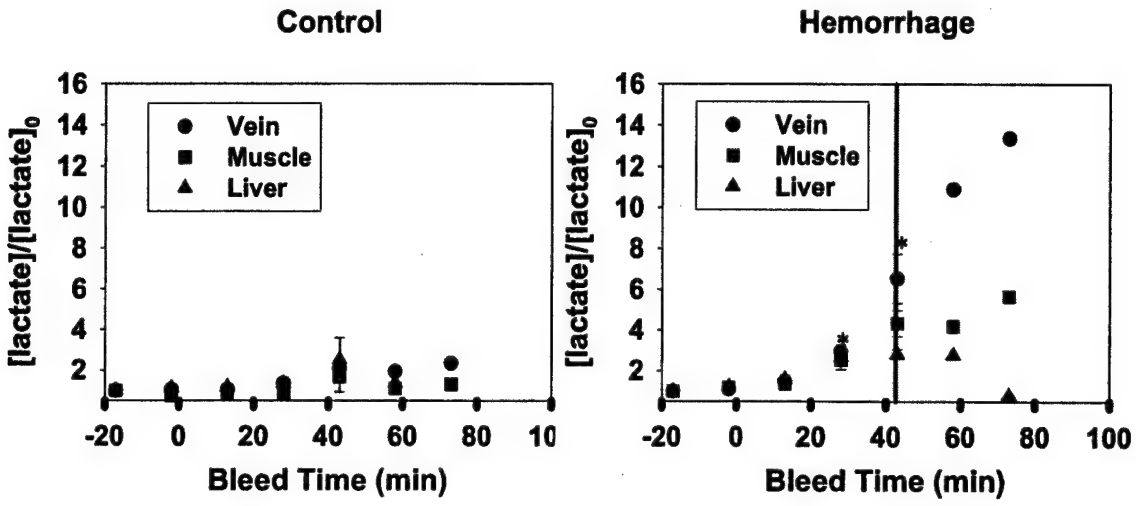

Figure 5. Relative lactate levels from microdialysis samples. Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.

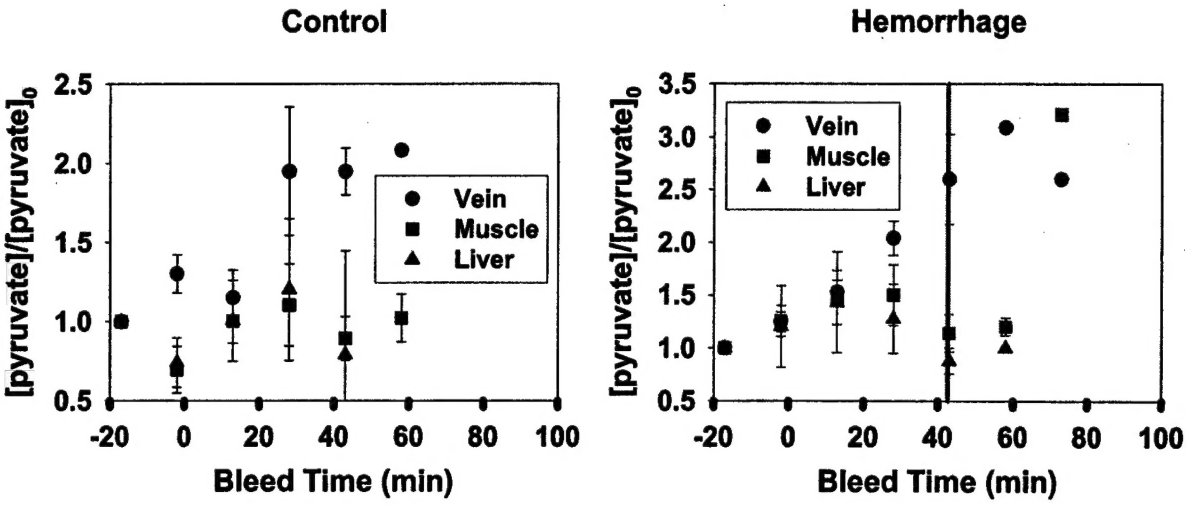

Figure 6. Relative pyruvate concentrations from microdialysis samples. Error bars represent ± one S.E.M. $*p < 0.05$ for hemorrhage versus control.

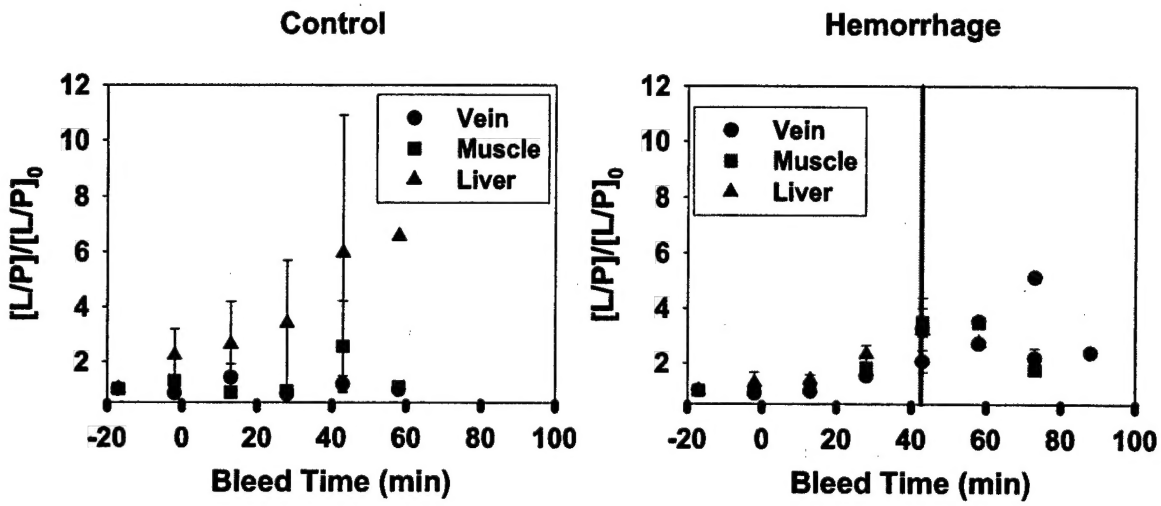

Figure 7. Relative lactate/pyruvate ratios from microdialysis samples. Error bars represent ± one S.E.M. $*p < 0.05$ for hemorrhage versus control.

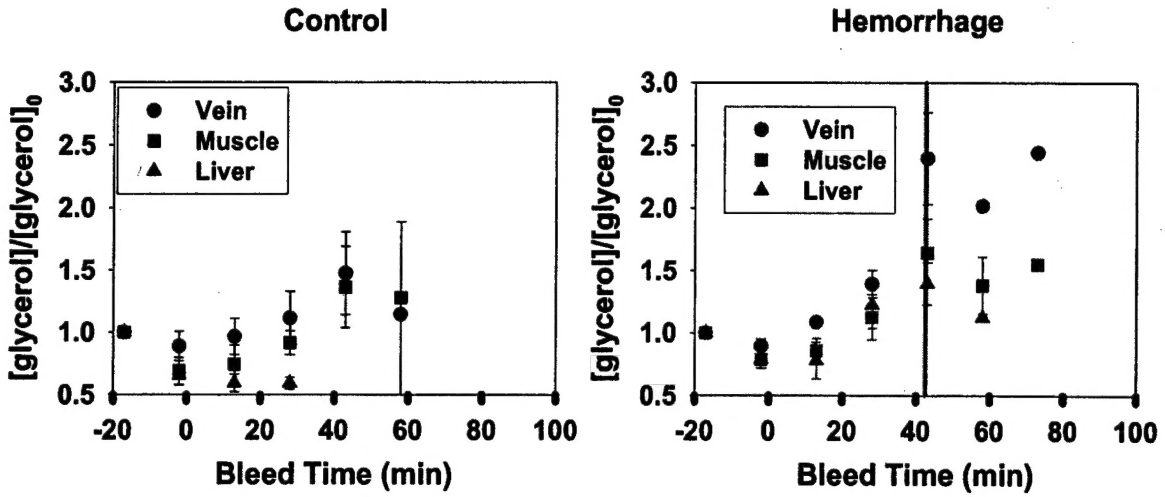

Figure 8. Relative glycerol concentrations from microdialysis samples. Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.

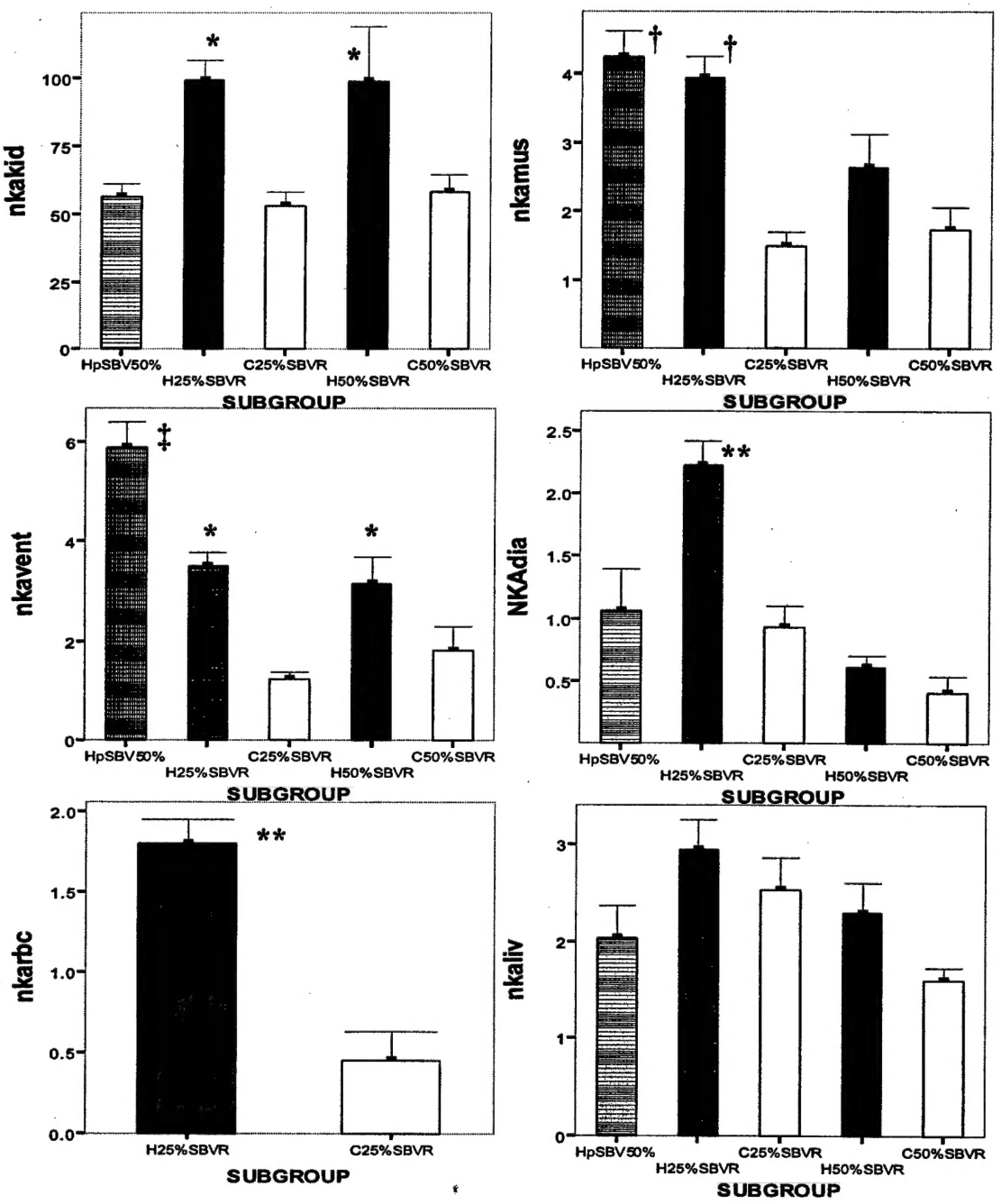

Figure 9.*Ex vivo* Na^+, K^+-ATPase (NKA) activity vs. stage of shock for various tissues. kid = kidney, mus = skeletal muscle, vent = heart left ventricle, dia = diaphragm, rbc = red blood cell, liv = liver. RBC results from early hemorrhage and H50%SBVR unavailable at this time. Error bars represent ± one S.E.M.

$^{*}p < 0.05$ vs. early hemorrhagic shock and controls. $^{\dagger}p < 0.05$ vs. H50%SBVR and controls. $^{\ddagger}p < 0.05$ vs. late hemorrhagic shock and controls. $^{**}p < 0.05$ vs. all other groups shown